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PRINCIPAL INVESTIGATOR: Ho Wang Lee, M.D.

CONTRACTING ORGANIZATION: Korean University Medical College
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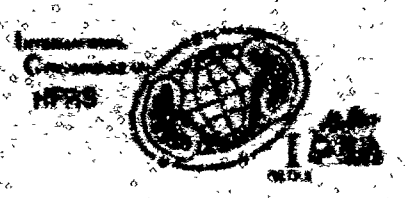
The 1st International Conference
on
Hemorrhagic Fever with Renal Syndrome

PROGRAM & ABSTRACTS



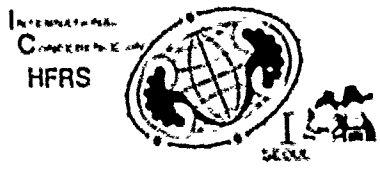
Seoul, Korea, May 4-6, 1990

Organized by
- WHO Collaborating Center for Virus Reference and Research (Hemorrhagic Fever with Renal Syndrome)
- The Institute for Viral Diseases, Korea University

Sponsored by
- The National Academy of Sciences, Republic of Korea
- WHO Western Pacific Region
- The Korean Society of Virology
- The Korean Society of Infectious Diseases
- US Army Medical Research Institute of Infectious Diseases, Frederick, MD
- US Army Medical Research Unit-Republic of Korea
- Korea Green Cross Corporation

The 1st International Conference on Hemorrhagic Fever with Renal Syndrome

PROGRAM & ABSTRACTS



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. US Army Medical Research Unit-Republic of Korea
. Korea Green Cross Corporation

= CONTENTS =

CHARIMAN'S WELCOME

Dear Colleagues,

It is a great privilege and pleasure for me as a chairman of the Organizing Committee to welcome you to the 1st International Conference on Hemorrhagic Fever with Renal Syndrome. Seoul, Korea.

An interesting and stimulating scientific program to update knowledge on hemorrhagic fever with renal syndrome and the exciting social program has been planned and we hope that you will actively participate in all programs.

The Organizing Committee and Secretariat have made every possible effort to insure that your stay in Seoul is comfortable, worthwhile, and memorable.

Welcome to Seoul and to the 1st International Conference on Hemorrhagic Fever with Renal Syndrome, and may we have a productive and enjoyable gathering.

Sincerely yours,

Ho Wang Lee
Chairman
Organizing Committee

ORGANIZING COMMITTEE

Chairman:	Ho Wang Lee
Co-chairmen:	Yong-Tae Yang and Joel M. Dalrymple
Secretary-General:	Pyung-Woo Lee
Treasurer:	Luck-Ju Baek
Planning:	In Whn Seong

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Gum-Ryong Kim	Suhnggwon Kim
Jung-Sang Lee	Yun-Tai Lee
Yong Ju Lee	Seung-Chul Park

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Y.- C. Chan (Singapore)
B. Niklasson (Sweden)
J. M. Dalrymple (U.S.A.)
D. C. Gajdusek (U.S.A.)
K. M. Johnson (U.S.A.)
E. A. Tkachenko (U.S.S.R.)
A. Gligic (Yugoslavia)

GENERAL INFORMATION

Opening Ceremony

All participants and accompanying persons are invited to attend the opening ceremony to be held in the grand conference room, 2nd Floor, the National Academy of Sciences, on May 4 (Thursday), 1989, at 0900 hours. The ceremony will be presided over by the Chairman of the Organizing Committee, Prof. Ho Wang Lee.

Speakers will be the Minister of Public Health and Social Affairs, the Honorouble Dr. Moon Tai Joon and the President of the National Academy of Sciences of the Republic of Korea, Dr. Ton-Kak Suh.

Social Program

The Organizing Committee sincerely welcomes all registered participants to the following acitivites at Palace Hotel. Admission is by invitation only (name badge or guest card required).

Pre-Conference Reception	**Wednesday, May 3, 1989** **at 18:00** (Cosmos Room, 12th Floor)
Welcome Party	**Thursday, May 4, 1989** **at 19:00** (Royal Ballroom, 1st Floor)
Farewell Party	**Saturday, May 6, 1989** **at 19:00** (Royal Ballroom, 1st Floor)

Conference Office and Registration

The Conference office hours are:

Wednesday, May 3, 1989	**1200-2100 hours** (Palace Hotel)
Thursday, May 4, 1989 **through the Conference**	**0830-1800 hours** (National Academy of Sciences)

The registration will take place at the desk, 1st Floor Lobby, Palace Hotel, beginning at 1200 hours, May 3(Wednesday) only. From May 4 (Thursday) to May 6 (Saturday) the registration desk will be at 2nd Floor, the National Academy of Sciences.

Name Badges

Access to Conference scientific sessions and to social functions is available only to those wearing name badges or holding personal invitations.

Currency and Banking

The Citizens Bank which is located near the Palace Hotel (50 m) will provide currency exchange and banking facilities for the Conference.

The bank is open from 0930 to 1630 hours from Monday to Friday, and 0930 to 1330 hours on Saturday.

Credit Cards

The Organizing Committee regrets that payments due to the Conference for fees, accommodation, tours, etc., cannot be made by credit card.

Dress

Dress for all social functions is informal.

Shuttle Bus between Palace and the National Academy of Sciences

For the convenience of Conference participants, a shuttle bus service will run between hotel and the National Academy of Sciences before and after the Conference.

Emergency Medical Services

Emergency Medical services may be arranged by the Secretariat. During off-hours, contact hotel front desk.

The Conference cannot accept responsibility for debts incurred by individuals as a result of illness or injury.

Smoking

Participants are asked to observe a Conference regulation against smoking in lecture room.

Post-Conference Tour (Optional)

The Organizing Committee has made arrangement for optional tour. Ticket for the tour can be purchased at the registration desk. Tour accommodations will be made on shared twin-room basis. Tour price includes room, all board and transport. Bus will be indentified with the appropriate tour members. The tour depart from Palace Hotel on May 7 (Sunday) at 9:00 a.m.

Mt. Soraksan National Park and Kyongju Tour
May 7-10, 1989 (3 nights and 4 days)
Cost: US$396 (twin use)
US$540 (single use)

Accompanying Persons' Program and Daily Seoul Vicinity Tours

The accompanying persons' program. Shopping in Itaewon will be planned on May 5 (Friday) afternoon.

Conference participants and family members who wish to take part in daily Seoul vicinity tours will be arranged at Information desk, Palace Hotel.

CONFERENCE CALENDAR

Time \ Date	May 3 (Wednesday)	May 4 (Thursday)	May 5 (Friday)	May 6 (Saturday)	May 7-10 (Sun.-Wed.)
8:00 AM	Arrivals	Registration			
9:00		Opening Ceremony			
10:00		Invited Lectures 1	Invited Lectures 3 and Free Communications 2	Free Communications 4 and Conclusion	Post-Conference Tour (Mt. Soraksan National Park and Kyongju Tour)
11:00					
12:00 Noon					
1:00 PM		Lunch	Lunch		
2:00		Free Communications 1 and Invited Lectures 2	Free Communications 3		
3:00					
4:00					
5:00					
6:00	Registration & Pre-Conference Reception				
7:00		Welcome Party		Farewell Party	
8:00					

SCIENTIFIC INFORMATION

Official Language

The official language of the Conference is English. Simultaneous interpretation will not be available.

Room for Presentations

All Conference sessions will be held in Grand Conference Room located on the 2nd floor of the National Academy of Sciences.

Audio-Vidual Aids

All slides must be handed in to the Audio-Vidual room at least one hour before the start of the session. Slide projectors will be available for final viewing. All sildes must be collected from the AV room as soon as possibl(after the presentation.

Late Abstracts

Participants should consult the Secretariat regarding the photo-copying of their abstracts for distribution at appropriate sessions.

Presentation

Speakers will be seated in the front row of the session room and must move quickly to the microphone during the individual introductions by the Session Chairmen. After the presentation, speakers should return to their seats and remain there until the commencement of the discussion period.

Timing

Speakers must abide by their assigned presentation time. The time for invited speaker is 20 minutes and for free communication is 15 minutes including discussion. If the Chairman is informed that a speaker can not make his presentation, the Chairman should announce the schedule change at the beginning of the session. And the speaker's assigned presentation time will be used for free discussion.

SCIENTIFIC PROGRAM

Thursday, May 4 (Morning)

INVITED LECTURES 1 (Grand Conference Room)

Chairmen: Y. T. Yang (Korea), Karl M. Johnson (U.S.A.)

IL1-1 10:00 *HFRS Past and Present: Overview*
D. C. Gajdusek (U.S.A.)

IL1-2 10:30 *The rat type epidemic hemorrhagic fever (EHF) in China*
G. Song (China)

IL1-3 10:50 *Haemorrhagic fever with renal syndrome in Southeast Asia*
Yow-Cheong Chan (Singapore)

Chairmen: J. H. Chun (Korea), Guido van der Groen (Belgium)

IL1-4 11:10 *The study of HFRS in the Soviet Union*
E. A. Tkachenko, S. G. Drozdov (U.S.S.R.)

IL1-5 11:30 *Hemorrhagic fever with renal syndrome in Yugoslavia*
Ana Gligić, M. Obradović, R. Stojanović, J. LeDuc, G. Diglisić, V. Lukač, D. Nastić (Yugoslavia, U.S.A.)

IL1-6 11:50 *Hemorrhagic fever with renal syndrome in Sweden*
Bo Niklasson, (Sweden)

IL1-7 12:10 *Epidemiology and distribution of Hantaviruses in the Americas*
James LeDuc, Thomas Kstazek, C. Rossi (U.S.A.)

12:30 Discussion

12:40 Lunch

Thursday, May 4 (Afternoon)

FREE COMMUNICATIONS 1 and INVITED LECTURES 2
(Grand Conference Room)

Chairmen: James LeDuc (U.S.A.), Song Gan (China)

FC1-1 14:10 *A review of HFRS in Slovenia*
Tatjana Avšič-Županc, B. Čižman, A. Kraigher, D. Ferluga (Yugoslavia)

Symposium Topics (cont'd)

FC1-2 14:25 *Some characteristic findings on the experimentally infected rats with Seoul virus*
Takahisa Yamanouchi, Kayoko Dohmae, M. Yasuda, K. Yamanishi, J. Kawamata, T. Kurata, H. Miyamoto, H. W. Lee (Japan, Korea)

FC1-3 14:40 *Epidemiologic studies of Hantaavirus infection among urban rats in Japan*
J. Arikawa, Mingyang Lan, Xian-Kui Zhang, Ikuo Takashima, Nobuo Hashimoto (Japan)

FC1-4 14:55 *Seroprevalence of antibodies to Hantaan virus among US Marines deployed to Korea*
Dale A. Carroll, Chong H. Hong, R. M. West, H. W. Lee (U.S.A., Korea, Japan)

FC 1-5 15:10 *Epidemiology and rapid diagnosis of nephropathia epidemica (NE) in Finland*
M. Brummer-Korvenkontio, Klaus Hedman, Eeva-Marjatta Salonen, Antti Vaheri (Finland)

15:25 Coffee break

Chairmen: E. A. Tkachenko (U.S.S.R.), Yow-Cheong Chan (Singapore)

FC1-6 15:45 *Evidence for hemorrhagic fever with renal syndrome at the Andaman and Nicobar Islands*
Guido van der Groen, Guy Hoofd, M. Bharadwaj, U. Chawla, S. Sehgal (Belgium, India)

FC1-7 16:00 *Hemorrhagic fever with renal syndrome in Germany*
Juergen Pilaski, Liv Bode, Ottmar Gorschewsky (W. Germany)

FC1-8 16:15 *The present state of Hantaavirus infection in Rome*
Mario Nuti (Italy)

IL2-1 16:20 *Review of Hantaavirus ultrastructure*
John D. White (U.S.A.)

IL2-2 16:50 *Inclusion bodies of Hantan (HFRS) virus expressed by recombinant vaccinia virus as revealed by IEM*
Hung Tao, Liu Hongmei, Zhou Jingyi (China)

17:10 Discussion

Friday, May 5 (Morning)

INVITED LECTURES 3 and FREE COMMUNICATIONS 2
(Grand Conference Room)

Chairmen: Joel M. Dalrymple (U.S.A.), C. Yong Kang (Canada)

IL3-1	9:00	*Coding strategies of S genomic segments of different members of Hantavirus genus* C. Yong Kang, Mark Parrington, Dongwan Yoo (Canada)
IL3-2	9:20	*Expression of the Hantaan M genome segment for recombinant vaccine development* Connie Schmaljohn, Jiro Arikawa, Hugh LaFenetiere, Yong-Kyu Chu, Joel Dalrymple, U.S.A.
IL3-3	9:40	*Field trial of HFRS vaccine in man* H. W. Lee, C. N. Ahn, L. J. Back, J. W. Song, S. C. Park, T. J. Seo, D. W. Kim (Korea)
FC2-1	10:00	*A clinical study of inactivated vaccine against hemorrhagic fever with renal syndrome in volunteers* Dong Jin Suh, Jin Won Song, Ho Wang Lee (Korea)
FC2-2	10:10	*Clinical trial of hemorrhagic fever with renal syndrome vaccine in volunteers* D. W. Kim, S. C. Park, K. H. In, J. W. Song, L. J. Back, H. W. Lee (Korea)
FC2-3	10:20	*Antibody responses of individuals vaccinated with HFRS inactivated vaccine* Yu Yong-Xin, Zhe Zhi-yong (China)
	10:35	**Discussion and Coffee break**

Chairmen: J. S. Lee (Korea), Junichi Kawamata (Japan)

IL3-4	10:50	*The pathophysiology of hemorrhagic fever with renal syndrome* Thomas M. Coseriff (U.S.A.)
IL3-5	11:10	*Comparison of different clinical forms of hemorrhagic fever with renal syndrome in the world* Juhani Lähdevirta (Finland)
IL3-6	11:30	*Outbreak and control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan* Junichi Kawamata, Takahisa Yamanouchi, Kayoko Dohmae, Hiroyuki Miyamoto, Michiaki Takahashi, Koichi Yamanishi, Tsuyoshi Kurata, H. W. Lee (Japan, Korea)

IL3-7 11:50 *Chemotherapy of HFRS*
John W. Hurditch, C.M. Halang, T.M. Cosgriff, M.Y. Guang, J.I. Smith, Z.A. Wu, J.W. LeDuc, Z.M. Zheng, J.M. Meegan, C.N. Wang, P.H. Gibbs, X.E. Gui, K.W. Yuan, T.M. Zhang, D.O. Oland, H. W. Lee (U.S.A., China, Korea)

12:10 Discussion

12:20 Lunch

Friday, May 5 (Afternoon)

FREE COMMUNICATIONS 3 (Grand Conference Room)

Chairmen: Ana Gligić (Yugoslavia), Juergan Pilaski (W. Germany)

FC3-1 14:00 *Molecular cloning and Nucleotide sequence analysis of the genome of nephropathia epidemica virus strain Hallnas B1*
L. H. Gielel, R. Stohwasser, E.K.E. Bautz, L. Zoller, G. Daral (W. Germany)

FC3-2 14:15 *Characterizations of Hantaan virus envelope glycoprotein antigenic determinants related to virus neutralization and pathogenesis in suckling mice*
Jiro Arikawa, Nobuo Hashimoto (Japan)

FC3-3 14:30 *Experimental infection of human vascular endothelial cells with Hantaviruses*
Richard Yanagihara, David J. Silverman, D. Carleton Gajdusek (U.S.A.)

FC3-4 14:45 *Studies on the propagation of Hantaan virus '76-118 in MDCK cell cultures*
Sung I. Chung, Yong T. Yang, Chul S. Choi (Korea)

FC3-5 15:00 *A promising approach for isolation of Hantavirus from HFRS patients during later course*
Dongyou Yan, X.S. Gu, Z.W. Jin, et al. (China)

FC3-6 15:15 *Isolation of hemorrhagic fever with renal syndrome virus from peripheral blood leukocytes of human patients*
Gum-Ryong Kim, Tai-Gyu Kim, Moon-Gun Rhyu and Byung-Uk Lim (Korea)

15:30 Coffee break

Chairmen: Y. T. Lee (Korea), Bo Niklasson (Sweden)

FC3-7 15:45 *Determination of IgM type of antibodies against Hantaviruses in sera of Dutch and Belgian patients with an acute form of hemorrhagic fever with renal syndrome (HFRS)*
Guy Hoofd, Jan Clement, A. Lefevre, B. Niklasson, G. van der Groen (Belgium, Sweden)

FC3-8 16:00 *Determination of IgM type of antibodies against Clethrionomys (CO 13991) and Apodemus type (DNT 76-118) of Hantaviruses in sera of Yugoslavian patients with hemorrhagic fever with renal syndrome (HFRS)*
Guy Hoofd, Tatjana Avšič-Županc, J. LeDuc, G. van der Groen (Belgium, Yugoslavia, U.S.A.)

FC3-9 16:15 *Rapid serodiagnosis of HFRS virus infection using high density particle agglutination, a preliminary report*
Tetsuo Tomiyama, Ho Wang Lee (Japan, Korea)

FC3-10 16:30 *Enzyme-linked immunosorbent assay using baculovirus expressed nucleocapsid protein*
Kazuyoshi Suetyama, Yoshiharu Matsuura, Hiroshi Ushijima, Takashi Kitamura (Japan)

FC3-11 16:45 *Serological comparisons of Hantavirus strains*
Joel Dalrymple, Yong-Kyu Chu, Sherman Hasty, James LeDuc, Connie Schmaljohn, Ho Wang Lee (U.S.A., Korea)

FC3-12 17:00 *Differential serologic diagnosis of hemorrhagic diseases among suspected HFRS in Korea in 1986*
L.J. Back, J. W. Song, H. W. Lee (Korea)

FC3-13 17:15 *Demonstration of presence of 5th serotype of Hantavirus: by interpretation of differential sero-diagnostic analyses of sera from HFRS patients*
P.W. Lee, H. W. Lee, G. van der Groen, Avšič-Županc (Korea, Belgium, Yugoslavia)

Saturday, May 6 (Morning)

FREE COMMUNICATIONS 4 and CONCLUSION

(Grand Conference Room)

Chairmen: Seung Chul Park (Korea), Richard Yanagihara (U.S.A.)

FC4-1 9:00 ***Clinical analysis of fatal cases in hemorrhagic fever with renal syndrome (HFRS)***
H. Y. Yoon, K. H. Kim, J.S. Han, S. Kim, J. S. Lee (Korea)

FC4-2 9:15 ***Successful Delivery in a patient with hemorrhagic fever with renal syndrome (HFRS)***
D. W. Chae, J. H. Earm, J.S. Han, J. K. Kim, J. W. Choi, S. Kim, J. S. Lee, H. W. Lee (Korea)

FC4-3 9:30 ***Clinical features of hemorrhagic fever with renal syndrome (HFRS) caused by Seoul virus infection: a clinical and laboratory study on 29 cases in Seoul in 1984***
K. S. Ihm, H. J. Pyo, S. C. Park, H. W. Lee (Korea)

FC4-4 9:45 ***Magnetic resonance images (MRI) of kidney in hemorrhagic fever with renal syndrome (HFRS)***
Y. O. Kim, S. H. Kim, J. S. Han, S. Kim, J. S. Lee (Korea)

FC4-5 10:00 ***Activation of plasma kallikrein-kinin system in hemorrhagic fever with renal syndrome (HFRS)***
J. S. Han, J. T. Cho, S. K. Lee, S. Kim, J. S. Lee, M. Lee (Korea)

10:15 **Coffee break**

Chairmen: Gum-Ryong Kim (Korea), Juhani Lachdevirta (Finland)

FC4-6 10:30 ***The changes of plasma atrial natriuretic polypeptide (ANP) level according to each clinical phases of Korea hemorrhagic fever***
Jeong Euy Park, Gwan Gyu Song, Heul Jung Pyo, Seung Chull Park (Korea)

FC4-7 10:45 ***Changes in lymphocyte subpopulations during HFRS***
Richard M. Lewis, Ho Wang Lee, Anthony F. See, David B. Parrish, Jung Sik Moon, Dai Jung Kim, Thomas M. Cosgriff (U.S.A., Korea)

FC4-8 11:00 ***Platelet aggregation and release in HFRS patients***
Richard M. Lewis, Ho Wang Lee, anthony F. See, David B. Parrish, Jung Sik Moon, Dai Jung Kim, Thomas M. Cosgriff (U.S.A., Korea)

FC4-9 11:15 ***Scintigraphic measurement of the changes of pulmonary vasculature in Korean hemorrhagic fever***
Dong Soo Lee, Dae Jung Kim, Myung Chull Lee, Chang-Soon Koh (Korea)

FC4-10 11:30 *Distinctive inclusion body in the hemorrhagic fever with renal syndrome virus-infected rats and culture cells*
Michio Kimura, Takahisa Yamanouchi, Kayoko Dohmae, Masahide Yasuda, Keiichi Yamanishi, Junichi Kawamata (Japan)

FC4-11 11:45 *Prospective study on Panhypopituitarism as a sequela of hemorrhagic fever with renal syndrome (HFRS)*
S. Kim, J.S. Han, J. S. Lee, M. Lee (Korea)

FC4-12 12:00 *Dynamic observation of blood system changes in epidemic hemorrhagic fever*
TM Zhang, J. W. Huggins, CM Hslang, T.M. Cosgriff, J.I. Smith (China, U.S.A.)

FC4-13 12:15 *Detection and clinical significance of creatine phosphate kinase isoenzyme in epidemic hemorrhagic fever*
TM Zhang, J. W. Huggins, CM Hslang, T.M. Cosgriff, J.I. Smith (China, U.S.A.)

CONCLUSION

12:30 *HFRS: End of the beginning, Beginning of the end?*
Karl M. Johnson (U.S.A.)

ABSTRACTS

IL 1-2

THE RAT TYPE EPIDEMIC HEMORRHAGIC FEVER(HFRF) IN CHINA

J. [illegible], Institute of Virology, Chinese Academy of preventive Medicine, Beijing 100052, China

Global distribution of the rat type HFRF virus has been demonstrated, but clinical disease occurred rarely outside Asia, and epidemic was only found in China. An etiologic, epidemiologic, and clinical characterization of this newly recognized form of HFRF had been made after its first outbreak in 1981. New epidemics of this type HFRF were found later on in other parts of China(Jiangsu, Liaoning, northern part of Shanxi, and Shandong Provinces), data relevant to its characterization have been accumulated. Futher serotyping of the HFRF virus strains isolated in China from various sources with panels of McAbs, and molecular analysis of the viral structure proteins(G_2 and NP) revealed distinctdifferences, both antigenically and molecularly between the rat type and the Apodemus type viruses. A brief overview of the present knowledge of the rat type HFRF in China is given.

IL 1-3

HAEMORRHAGIC FEVER WITH RENAL SYNDROME IN SOUTHEAST ASIA

You-Cheong CHAN, National University of Singapore, Singapore

Serological evidence of human and rodent infections suggests that hantaviruses, the cause of haemorrhagic fever with renal syndrome, have a worldwide distribution. Indirect fluorescent antibodies to Hantaan and/or Seoul viruses have been found in humans and urban rats in many countries in Southeast Asia including Burma, Malaysia, Philippines, Singapore and Vietnam. Human illness caused by these viruses has recently been documented in Malaysia where hepatic dysfunction has been found to be a prominent clinical finding. In Singapore, a virus antigenically related to Seoul virus has been isolated from Rattus norvegicus. The present status of hantaviruses and the clinical illness they cause in man in Southeast Asian countries are discussed.

IL 1-4

THE STUDY OF HFRS IN THE SOVIET UNION

E.A. Tkachenko and S.G. Drozdov, The Institute of Poliomyelitis and Viral Encephalitides, Ac. Med. Sci. of the U.S.S.R.

During last years the large-scale investigations on the etiology immunology epidemiology epizootology and clinic of HFRS in the U.S.S.R. were conducted. It was shown wide spread of the natural foci of HFRS in the U.S.S.R. since 1978 to 1988 about 50 thousands of HFRS cases were registered in 46 administrative regions. In far-eastern foci HFRS cases are more severe as compared with the cases in western foci. The lethality is not exceed more 3% in european and 10-15% in Far-East of the U.S.S.R. HFRS virus antigen has been found in lung tissue of more than 30 species of wild small animals which related to 6 Families and to 2 Orders (Rodentia and Insectivora). 30 HFRS virus strains were isolated from lungs of 8 species of rodents and from HFRS patients' blood and organs post mortem. IFA and RIPA analysis of HFRS virus strains were shown the existence 5 antigenical variants circulated among wild small animals in the U.S.S.R. It was proposed to use the figures to designate HFRS antigenical types instead of the name of rodents genus.

IL 1-5

HEMORRHAGIC FEVER WITH RENAL SYNDROME IN YUGOSLAVIA

Ana GLIGIĆ[1], Mirčeta OBRADOVIĆ[2], Radivoje STOJANOVIĆ[2], James LEDUC[3], Gordana DIGLISIĆ[1], Veselin LUKAČ[2], Dragoljub NASTIĆ[1], [1]Institute of Immunology & Virology, Belgrade; [2]Military Medicaly Academy, Belgrade; [3]US Army Medical Research Institute of Infect. Diseases, Fort Detrick, Frederick, USA.

During the period of 1952-1989, 878 cases of HFRS were described in Yugoslavia. Clinics' picture varied from mild to severe form with total lethality of 5.2%. Lethality increased from west to east from 0-33%. 662 patients' sera who contracted diseases from 1967 to 1989 were investigated by IFA test. Hantavirus antibodies were confirmed in 351 patients. 164 out of 446 investigated patients had IgM antibodies (Hantaan 76-118) in ELISA test.

Determination of areals and HFRS nosoareals was performed by serological discovering of antigens in tissues and antibodies in human and animal sera. Immune status of healthy population varied from 0 to57.3% but in endemic focci it was 21.9%.

In Yugoslav HFRS focci, antigen and antibodies were proved in 10 species of small mammals. Infection average frequency during epidemic in 1986 was 28.4%, but before epidemic it was 6.2%. Considering numerous population of species and level of infections, mammals had important place in epizootic process. Serological confirmation and virus isolation from A. flavicollis and Cl. glareolus proved that in small mammals population in Yugoslavia focci circulated at least 3 serotypes of hantaviruses. That is in correlation with different clinical picture and serological findings.

IL 1-6

HEMORRHAGIC FEVER WITH RENAL SYNDROME IN SWEDEN

Bo NIKLASSON, National Bacteriological Laboratory and the Swedish Defense Research Establishment, FOA-5, Stockholm, Sweden

The clinical disease of hemorrhagic fever with renal syndrome (HFRS) is called Nephropathia epidemica (NE) in Sweden. The etiological agent, Puumala virus (PUU), was isolated in 1984, fifty years after the original clinical and epidemiological description of this disease. The number of clinical cases of NE varies in a cyclic fashion with peaks every 3-4 years corresponding with the abundance of the vector Clethrionomys glareolus. Puumala virus has been compared with other strains of HFRS using immunoprecipitation and indirect immunofluorescence tests. ELISA for detection of PUU virus specific IgG and IgM have been developed and evaluated for both sero-epidemiology and patient diagnosis. The clinical spectrum of serologically confirmed cases range from the typical symptoms of fever, abdominal pain and renal affection to hemorrhagic manifestation. However, hemorrhagic manifestations are rare and no fatalities have been recorded. To study the ecology of PUU virus infection, different strains of wild, small mammals have been colonized and evaluated for their susceptibility to PUU virus.

IL 1-7

EPIDEMIOLOGY AND DISTRIBUTION OF HANTAVIRUSES IN THE AMERICAS

James LeDUC, Thomas KSIAZEK and C. ROSSI, USAMRIID, Ft. Detrick, Frederick, Maryland, U.S.A.

Two hantaviruses have been isolated from rodents captured in the Americas, Prospect Hill virus, a strain thought to be non-pathogenic, and Seoul-like viruses, which have been associated with human hemorrhagic fever with renal syndrome in Asia. Seoul-like virus isolates have been made from domestic rats captured in several cities in the USA, and from Belem, Brazil. Serosurveys found rats with anti-hantaviral antibodies common in many cities in the Americas, and there is strong evidence to suggest that Seoul-like viruses are widespread among rodent populations throughout the region. Limited attempts have been made to document human hantaviral infections in the Americas. Serosurveys of humans found antibody to hantaviruses among residents of the USA, Brazil, Bolivia, Uruguay and Argentina. Data remains fragmentary as to the extent of HFRS in the Americas, but overt disease, currently undiagnosed, probably exists.

Examination was recently completed of sera obtained from UN Forces during the Korean Conflict by the Hemorrhagic Fever Commission. A total of 240 patients were enrolled in this study based on a clinical diagnosis of Korean hemorrhagic fever between 1952 and 1954. Sequential sera were obtained at admission, about one week post-admission, and at discharge. These sera were examined by enzyme immunoassay for IgM and IgG anti-hantaviral antibodies, and all but 15 patients developed specific anti-hantaviral antibodies.

Thursday, May 4 (Afternoon)

FC 1-1

A REVIEW OF HFRS IN SLOVENIA

Tatjana AVŠIČ-ŽUPANC[1], B. Čižman[2], A. Kraigher[3], D. Ferluga[4]

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4 Institute of Pathology, Medical Faculty, Ljubljana

In Slovenia, the North-West part of Yugoslavia, the presence of Hantavirus disease has been reported. 24 clinically well documented cases of mild or severe forms of the disease were serologically confirmed. Using the IFA-test the prevalence of IgG antibodies against different Hantaviral antigens was demonstrated in patients sera and three different reactivity patterns were observed. Majority of the IFA positive human sera were confirmed by the Immuno blot method.

In two natural foci of HFRS where clinical documented cases were reported the distribution of Hantaviral infections was examined in small mammals. Simultaneous circulation of two Hantaviral type among free living small mammals were observed: Apodemus type and Clethrionomys type. This correlated well with different reactivity patterns of patients sera, different clinical picture of the disease and with the distribution of HFRS natural foci where the first or the second serotype is predominant.

FC 1-2

SOME CHARACTERISTIC FINDINGS ON THE EXPERIMENTALLY INFECTED RATS WITH SEOUL VIRUS.

Takahisa YAMANOUCHI [1], Kayoko DOHMAE [1], Masahide YASUDA [1], Koichi YAMANISHI [1], Junichi KAWAMATA [1], Takeshi KURATA [2], Hiroyuki HIYAMOTO [3] and Ho Wang LEE [4], [1] Research Inst. for Microbial Dis., Osaka Univ., Osaka, [2] NIH, Tokyo, [3] Wakayama Med. College, Wakayama, Japan; [4] Inst. Viral. Dis., Korea Univ., Seoul, Korea.

Persistence of antibody against Hantaan virus in convalescent serum of a patient, who was suffered from hemorrhagic fever with renal syndrome (HFRS) more 35 years before, was proved on immunofluorescent antibody assay (IFA) by H. W. Lee, in 1979. In 1988, the same human sera had reciprocal IFA titers of 256 ~ 512. In experimentally infected rats, seoul viruses was isolated from brain, lung and other organs on the Vero E6 cells on more 100 weeks after inoculation.

In the contrary, the spontaneous and experimental eradications of seoul virus from rat-tumor (malignant fibrous histiocytoma) contaminated with the virus, were observed during the serial transplantation of tumor through the rats. The normal rats had never converted to seropositive against seoul virus during the long breeding in one cage mixed with strong seropositive rats experimentally infected with seoul virus, B-1 strain.

The infection from rats contaminated with seoul virus to human and the spreading viruses among the rats may depend upon the unknown factors in laboratory animal experimentation.

The laboratory type infection of HFRS may be typical infectious disease depended upon the relationship between host, parasites and environmental factors.

FC 1-3

EPIDEMIOLOGIC STUDIES OF HANTAVIRUS INFECTION AMONG URBAN RATS IN JAPAN

Jiro ARIKAWA, Mingyang LAN, Xian-Kui ZHANG, Ikuo TAKASHIMA and Nobuo HASHIMOTO,
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Epidemiological surveys of hantavirus infection among urban rats were repeated for ten times at a dumping ground area in Kamiiso town, Hokkaido, Japan in 1983-1988. Sero-positive rats were continuously obtained every survey and Seoul-like viruses were isolated in 1983, 1985 and 1988, but no patient was reported in this area.

Among adult rats (more than 4-moth-old), antibody prevalence, mean IFA titers and antigen positive ratios in tissue increased with age of rats. However, young rats represented only small number of sero-positive cases with low IFA antibody titers, but no antigen positive case was obtained. This results indicate that maternal antibody may protect from infection effectively.

Ectoparasites (*Laelaps echidninus* and *L. nuttalli*) were obtained from sero-positive rats. Attempts were made to isolate hantavirus by injecting their homogenates to suckling rats. Four weeks after inoculation, some of the rats produced antibodies to Seoulvirus.

FC 1-4

SEROPREVALENCE OF ANTIBODIES TO HANTAAN VIRUS AMONG US MARINES DEPLOYED TO KOREA

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Hemorrhagic Fever with Renal Syndrome (HFRS) is a significant problem for military units operating in the Republic of Korea. A prospective study was undertaken to determine the rate of seroconversion and incidence of subclinical HFRS among US Marines deployed to Korea between January and October 1988. No seroconversions were documented during 916.7 person/months of exposure. Three marines were positive for antibodies at the time of the initial bleed. One had been previously deployed to Korea the other two had not. None had a history of illness consistent with HFRS. Time of exposure ranged from 13 days to 245 days. Eleven percent of rodents trapped in the area of the camp were positive for antibodies to Hantaan virus. Eighty-six marines completed a post deployment survey to determine dust and rodent exposure. Eighty-one percent of the Marines reported significant dust exposure (greater than 33% of the time); while 42% reported rodent exposure in their work area and 23% in their sleeping area. This study illustrates that simple preventive measures may decrease the risk of HFRS in military units operating in the Republic of Korea.

FC 1-5

EPIDEMIOLOGY AND RAPID DIAGNOSIS OF NEPHROPATHIA EPIDEMICA (NE) IN FINLAND

Markus BRUMMER-KORVENKONTIO, Klaus HEDMAN, Eeva-Marjatta SALONEN, Antti VAHERI. Department of Virology, University of Helsinki

The first NE-positive bank voles were collected in 1977 in Puumala, Lake-Finland and the Puumala virus was isolated at the Department of Virology, University of Helsinki. This laboratory is the only place for NE serology in Finland, and the routine diagnostic service has been available for all Finnish hospitals and physicians. During the years 1980-1989 over 20 000 serum samples have been studied by IFAT, among them 7 000 paired sera. A total of 1 000 serodiagnoses have been made. Epidemiological data based on these 1 000 serologically confirmed cases will be presented. In addition, there were over 1 000 serum pairs with high titer, mostly representing recent NE cases. We have also developed an HI test (using Tween-ether treated Puumala virus antigen) for epidemiological prevalence studies of human and animal populations (Puumala-antigen can also detect Hantaan antibodies). In our laboratory IFAT has been the basic test for routine serodiagnosis of NE. Because IFAT detects antibodies already at the onset of the disease, we have established a "Rapid NE test" and sera of altogether 5 000 patients have been studied during the last 2.5 years. We recently developed a new test which is based on measurement of the antigen-binding avidity of the IgG antibodies. Another new test that we have developed for detection of acute Puumala virus infection is based on detection of IgM antibodies in patient sera using immunoblotting with purified Puumala virus in an immunoperoxidase reaction. These tests can provide a rapid diagnosis from single serum samples.

FC 1-6

EVIDENCE FOR HEMORRHAGIC FEVER WITH RENAL SYNDROME AT THE ANDAMAN AND NICOBAR ISLANDS

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At the end of 1988, four human sera originating from Andaman and Nicobar Islands in the bay of Bengalen were tested in the indirect immunofluorescent antibody assay (IFA) for the presence of IgG type of antibodies against Hantaviruses, the etiologic agents of hemorrhagic fever with renal syndrome (HFRS). One serum (No 3320) was positive in IFA with low and equal titers (1/32 - 1/64) when tested on Apodemus, Clethrionomys, Microtus and Rattus type of hantaviruses. This result was confirmed when tested in Western blot using Hantaan 76-118 as antigen. The IgM ELISA with CG 13891 as antigen (Clethrionomys type of Belgian origin) was positive (OD serum/OD cut off: 1.5).
The same serum was negative for leptospirosis and weak hemagglutination inhibition titers (in between brackets) were observed for JE (1/160), WN (1/10), DENG 2 (1/20) and CHIK (1/10). The case No 3320 in question was a 50 year old male, being a field worker and living in a rural area. The serum was taken 2 days after the onset of fever. The main symptoes being fever with haemoptysis. To our knowledge this is the first proof of an acute HFRS case at the Andaman and Nicobar Islands. Further investigations are in progress.

FC 1-7

HEMORRHAGIC FEVER WITH RENAL SYNDROME IN GERMANY

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Between July 1985 and May 1988 a total of 12 clinical HFRS cases has been observed in the southwestern part of Germany. 11 patients had shown severe renal failure and had to be hospitalized. For serological examination the indirect immunofluorescence antibody (IFA) technique was applied using Vero E6 cells infected with hantavirus strains 76-118, NE-Hällnäs, NE-Puumala, CG 18-20, and SR-11. IFA titers (IgG) ranged between 1 : 512 and 1 : 4096. In 5 sera also IgM titers could be demonstrated by ELISA. 8 sera were tested by Western Blot analysis. All reacted with a polypeptide with molecular weight of 50 kd. Hantavirus antibody titers could also be demonstrated in 4 different rodent species, i.e. in Rattus norvegicus, Clethrionomys glareolus, Microtus arvalis and Mus musculus.

This study was supported by BMFT grant 0318973 A.

FC 1-8

THE PRESENT STATE OF HANTAVIRUS INFECTION IN ROME

Mario MUTI - Dept. of Tropical Diseases, 1st University of Rome, Italy.

In Italy the first data on the presence of Hantavirus infection were recorded at the end of 1984, when Hantaan antibodies were found in 2.5% of inhabitants of Rome. In order to better clarify the epidemiology of Hantaan infection in Rome, where it has not yet possible to identify any human cases of HFRS, a study of 102 rodents trapped in urban and suburban areas was carried out. Antibody to Hantaan serotype were found in R.norvegicus (52%), R.rattus(17%) and M.m.domesticus (19%). The comparative study of antibody titers showed higher titers to Seoul serotype compared to Y 2508 strain or Hantaan serotype. The screening of dialized patients and high risk subjects (trappers, mammologists, boatmen, river policemen) showed different results. None of of the 66 trappers, 58 boatmen and 12 river policemen tested showed Hantaan antibody, while only 2 out of 20 mammologists presented antibody at low titer (1:32). Among the 51 dialized patients the antibody prevalence was 5.9%, a value not dissimilar to that recorded in a rural area north of Rome (5.6%). Considering subjects at risk with activity on the Tiber river, i.e. trappers, boatmen, river policemen the IFI test for Hantaan antibody was negative, in contrast to the test for Leptospiral antibody (MAT) that showed a positivity ranging from 10% (boatmen) to 21% (trappers).

IL 2-1

REVIEW OF HANTAVIRUS ULTRASTRUCTURE John D. White, Pathology Division, USAMRIID, Fort Detrick, Frederick, MD 21701-5011, USA

Isolation of an etiologic agent for HFRS was first reported in 1978 by Lee et al[1]. Observations by electron microscopy led to a conclusion that the virus most likely belonged in the family Bunyaviridae.[2,3] Biochemical analyses confirmed this and showed that these viruses have three-segmented, single-stranded, RNA genomes with a common 3' terminal sequence.[4] All Hantaviruses are serologically related and can be separated into at least four antigenic groups.[5] They have identical structure, a unit membrane with surface projections in a reticular pattern. Projections were hollow cylinders approximately 10 nm long. Hantaan and Seoul viruses were more uniform in shape and smaller than either Puumala or Prospect Hill viruses. Dimensions ranged from 100 nm (Maagi and Leaky isolates) to more than 200 nm in the most variable shaped viruses (Puumala virus).

We have used *Hantavirus*-specific antibodies and the corresponding gold-conjugated anti-IgG for immunolabeling. Viral particles and a tubular structure reacted specifically with polyclonal immune sera and monoclonal antibodies for Hantaviral envelope glycoproteins G1 and G2 but not with monoclonal antibodies for nucleocapsid antigen. Hung and coworkers[6] described virus-associated antigen on luminal surfaces of vesicles and plasma membranes of infected cells. Three types of inclusion bodies were virus-specific by immune electron microscopy. We have seen these structures also in a study of 48 isolates representing each of the serotypes.

(1) Lee, et al., J Infect Dis, 137:298-308, 1978; (2) McCormick, et al., Lancet, I:765-768, 1982; (3) White, et al., Lancet, I:768-771, 1982; (4) Schmaljohn and Dalrymple, Virology, 131:482-491 1983; (5) Lee, et al., J Clin Microbiol, 22: 940-944, 1985; (6) Hung et al., Intervirology, 23:97-106, 1985.

IL 2-2

INCLUSION BODIES OF HANTAN (HFRS) VIRUS EXPRESSED BY RECOMBINANT VACCINIA VIRUS AS REVEALED BY IEM

Hung Tao, Liu Hongmei and Zhou Jingyi, Institute of Virology, Beijing.

In studying morphogenesis of HFRS virus, we have described some distinctive morphogenetic features in cells infected with HFRS viruses. The intracytoplasmic inclusion bodies (Ib) have been the most characteristic of them. The present paper reports our preliminary results in identifying the core antigen expressed by vaccinia virus in 143 cell (a human myeloma cell line).

The cDNA of S(RNA) segment of Hantan virus* was inserted into vaccinia virus (TianTan Strain). Under the control of 7.5kb promotor, core antigen of HFRS virus was successfully expressed. The product of the expression was identified by both immunological and morphological parameters, HFRS positive in ELISA and immune colloidal gold EM.

Morphologically, the expressed antigen was abundant, typical inclusion bodies marked with immune gold particles were often seen independently located in cytoplasm or around factories of vaccinia virus, at the meantime, the normal morphogenetic process of vaccinia virus was evidently visiulized.

* donated by Dr. C.S.Schmaljohn

FC 2-1

A CLINICAL STUDY OF INACTIVATED VACCINE AGAINST HEMORRHAGIC FEVER WITH RENAL SYNDROME IN VOLUNTEERS.

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Formalin inactivated purified Hantaan virus vaccine from suckling rat brains was supplied from Mock Am Research Institute, Green Cross corporation, Korea. 5120 unit by ELISA test of vaccine per ml was administered subcutaneously into 41 volunteers. 30 days after vaccination antibody response was measured by indirect immunofluorescence technique and ELISA test. General symptoms and local reactions were recorded by claim from vaccinees at the same time. Seroconversion rate after primary injection was 93% (38/41). Development of IgG antibody tested by immunofluorescence technique after primary injection (93%) was better than IgM antibody formation by ELISA test (85 %). Side reactions such as itching, induration, swelling or fever developed transiently in some vaccinees. This is a preliminary report on vaccination with inactivated Hantaan virus vaccine against volunteers and further study of anamnestic antibody response after booster vaccination is in progress.

FC2.3

CLINICAL TRIAL OF HEMORRHAGIC FEVER WITH RENAL SYNDROME VACCINE IN VOLUNTEERS

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The Institute for Viral Disease, Korea University

Formalin inactivacted purified Hantaan virus vaccine from suckling rat brain was supplied from Mok-Am Research Institute, Korea Green Cross Cooperation. 5,120 units/ml vaccine by ELISA test was administered subcutaneously into volunteers.
Antibody responses after vaccination was measures by IFAT and ELISA test. General symptoms and local reactions were recorded by claim from vaccinee 30 days after vaccination.
Seroconversion rate of vaccinee after primary injection of vaccine 5,120 units by subcutaneous route was 28/35(80%).

This is preliminary report of vaccination with formalin inactivated suckling rat brain Hantaan virus vaccination against volunteers and anamnestic responses after booster injection is in progress.

FC 2-3

Antibody Responses of Individuals Vaccinated with HFRS Inactivated Vaccine.

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A lot of HFRS virus vaccine was prepared with M. Gerbil kidney tissue culture, using Z]0 strin (type]) and inactivated by 0.05% b-propiolactone.

No live virus was detected in the vaccine after 3 passages of it in Vero-E6 cells. Rabbits produced neutralizing antibodies (40-]60) and HI ()0-20) to HFRSV after twice injections with the vaccine.

Ten volunteers, 7-25 years of age with HFRSV anti body negative, were selected for trial. On day 0,7, and 21, each person was injected with 1ml vaccine by intramuscularly. After immunization, 4 vaccinees developed neutralizing antibodies on 28th day (2 doses) and 9 developed N and HI antibodies on 42th day (3 doses). None of these vaccinees developed fever or other local and general side reactions.

This results indicated that this inactivated HFRS vaccine appears to be safe and immunogenic for humans.

IL 3-1

CODING STRATEGIES OF S GENOMIC SEGMENTS OF DIFFERENT MEMBERS OF HANTAVIRUS GENUS

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We have cloned and sequenced the genomic S RNA segments of Hantaan virus strain 76/118 (HTV) and Prospect Hill virus (PHV). These two viruses are members of the hantavirus genus in Bunyanviridae and were isolated from two different animals in two different continents. The S genomic segments of HTV and PHV have 1696 and 1675 nucleotides respectively. There is approximately 57% nucleotide sequence homology between the S segments of the two viruses. Both S genomic segments of HTV and PHV have one large open reading frame in the viral complementary sequence capable of coding for 429 and 433 amino acid long nucleocapsid proteins for HTV and PHV respectively. The overall amino acid sequence homology between the two nucleocapsid proteins is approximately 62%. A higher degree of amino acid sequence homology is found in the hydrophobic regions of the proteins. Furthermore, 83% of the last 123 amino acids at the C termini are homologous between the two virus nucleocapsid proteins. These results clearly explain the immunologic cross-reactivity between the two strains of hantavirus in the absence of cross hybridization between cDNA clones of the S RNA genomes. There are higher degrees of conservation on the amino acid sequences than on the nucleotide sequences between the two viruses. These results indicate that conservation of functional domain(s) of the protein is more important than the nucleotide sequence conservation in order to maintain the infectious nature of the virus.

IL 3-2

EXPRESSION OF THE HANTAAN M GENOME SEGMENT FOR RECOMBINANT VACCINE DEVELOPMENT

Connie SCHMALJOHN, Jiro ARIKAWA, Hugh LaPENOTIERE, Yong-Kyu CHU and Joel DALRYMPLE, USAMRIID, Ft. Detrick, Frederick MD, U.S.A.

In a continuing effort to develop a recombinant DNA vaccine for HFRS, we have used two eucaryotic viral vectors, vaccinia virus and a baculovirus (*Autographa californica* nuclear polyhedrosis virus), to express the Hantaan viral genes encoding the envelope glycoproteins (G1 and G2). Recombinant viruses were prepared that contained the complete M segment of Hantaan virus or coding regions of the M segment representing only G1 or G2. Both expression systems produced proteins indistinguishable from authentic Hantaan viral proteins when examined by polyacrylamide gel electrophoresis, fluorescent antibody staining, and ELISA with a variety of polyclonal and monoclonal antibodies. Mice and rabbits immunized with recombinants containing the entire M segment developed neutralizing antibody responses. Hamsters were immunized with vaccinia-Hantaan M recombinants and later challenged with Hantaan virus. Serum antibody titers were measured by IFA and ELISA, and lung sections were examined for the presence of viral antigen. Immunized hamsters appeared to be protected from infection with Hantaan virus, but control animals immunized with vaccinia recombinants containing Hantaan S segment cDNA seroconverted and had antigen in their lungs.

To examine the potential usefulness of a recombinant vaccine against rat-type hantaviruses, we cloned and sequenced the M segment of SR-11 virus and compared it to that of Hantaan. Approximately 75% of the amino acids comprising G1 and G2 were conserved between the two viruses, demonstrating a molecular basis for the observed serologic cross-reactivity, and raising the possibility that a single vaccine might be constructed to protect against multiple hantaviruses.

Subject classification
Vaccine, Oral with slide projector

IL 3-3

FIELD TRIAL OF HFRS VACCINE IN MAN

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Antigenicity of Hantaan virus (ROK84-105) and Seoul virus (80-39) harvested from suckling rats brains was better than the viruses grown in Vero E6 cell cultures in S.D. rats, and antigen titers were 40,960 units/ml by ELISA. Infectivity of Hantaan and Seoul virus was completely inactivated with 0.05% formalin at 4°C for 10 days. The vaccine was made by the modified method of Japanese encephalitis mice brain vaccine and antigen titers of the inactivated purified vaccines was 20,480 units/ml by ELISA. Protein content of the vaccine was 27-50 μg/ml. Primary and secondary antibody responses were demonstrated in rats after administration of 512 and 128 units of antigen IM, respectively. Vaccinated Apodemus mice were completely immune after challenge with 1,000 SM LD_{50} of live Hantaan virus I.M. Following results were obtained in human trial of Hantaan virus vaccine in volunteers in Korea. Seroconversion rate and antibody titers of vaccinee following S.C. inoculation of the vaccine is better than I.M. inoculation. Primary antigenic dose of the vaccine S.C. was 5,120 units and seroconversion rate following one shot was 112/125 (89%) but all of 30 persons who received two doses of the vaccine at 1 month interval was seropositive (100%) by IFAT and ELISA test. Occurence of general symptoms and local reactions of vaccinee after SC inoculation were higher than IM inoculated vaccinee and the details will be discussed. According to the results, the vaccine is safe and effective after inoculation of two doses S.C. against Hantaan virus infection in man.

IL 34

THE PATHOPHYSIOLOGY OF HEMORRHAGIC FEVER WITH RENAL SYNDROME.

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Any explanation of the pathophysiology of hemorrhagic fever with renal syndrome (HFRS) must take into account the complicated course of the disease, which includes the sequential occurrence of fever, hypotension, and renal failure, as well as variable degrees of hemorrhage. As with other infections, fever is probably produced by biological mediators, such as interleukins, interferons, and tumor necrosis factor, although these mediators have not been measured in HFRS patients. There is very good evidence that hypotension is a consequence of vascular dysfunction, including increased vascular permeability and inappropriate vasodilation. Renal failure does not appear to be a consequence of systemic hypotension, but rather appears to result from intrarenal pathology. Hemorrhage is a consequence of vascular-platelet dysfunction and of intravascular activation of coagulation.

IL 3-5

COMPARISON OF DIFFERENT CLINICAL FORMS OF HEMORRHAGIC FEVER WITH RENAL SYNDROME IN THE WORLD

Juhani Lähdevirta, Aurora Hospital, Helsinki, Finland

The clinical entirety called Hemorrhagic Fever with Renal Syndrome (HFRS) has originally contained two categories: The severe form of Korean Hemorrhagic Fever (KHF) type and the mild form of Nephropathia epidemica (NE) type in Scandinavia. By definition both these extremities of HFRS have as main manifestation the acute tubular, interstitial and hemorrhagic nephritis which are histologically very identical. KHF and NE are viral and general infections presenting also other organ manifestations, such as carditis, hepatitis and meningoencephalitis. The difference in the severity appears mainly in the mortality (5-10% in KHF vs. 0.1-0.3% in NE) and in more pronounced hemorrhagic manifestations in KHF.

The increasing experience from different countries and continents, mainly in East and North Asia and Europe has revealed clinical diseases caused by Hanta-viruses which seem to be identical with KHF or NE or with intermedium severity. Possibly there is a spectrum of clinical severity according to the pathogenity of different viruses or strains. The clinical comparison is rendered by differencies in clinical and laboratory praxis in various countries. In certain degree it could be improved by international adjustements of clinical and laboratory findings and clinical staging.

The differencies in epidemiology, having the second interest, obviously belong to the environmental differencies which influence the ways of transmission.

IL 3-6

OUTBREAK AND CONTROL OF LABORATORY ACQUIRED HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) IN JAPAN

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By the end of 1985, 126 human cases of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) were recorded in Japan. Close relationship was observed between high IFA titers against HFRS viruses of laboratory rats and occurrence of HFRS patients in a laboratory animal facility. Laboratory animal experimentor contracted HFRS more frequentry than laboratory animal technicians or caretakers. Inhalation of HFRS-virus highly contaminated air is the likely causes of infection. Wound infection during animal experiments may be another important route of infection. Transfer of infected laboratory rats from an animal facility to the other animal facility may cause spreading of HFRS infection. Causative viruses of HFRS were isolated from experimentally induced, transplantable animal tumor. Thus, tissue fragments or cells of animal tumors are a potential source of spreading the HFRS virus. Eradication of HFRS virus from a contaminated animal facility can be achieved best by elimination of all animal in an infected colony. In some cases, however, infection apparently disappeared without instituting particular control measures other than ordinary procedures for care and management of laboratory animals.

IL 3-7

CHEMOTHERAPY OF HFRS

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Attempts to provide specific chemotherapy of HFRS have included clinical trials of ribavirin, interferon, poly ICLC, cytoxan and acyclovir. Ribavirin has proven effective in controlled clinical trials, both against the arenavirus, Lassa fever; and the bunyaviruses, sandfly fever, Sicilian and HFRS. It has broad-spectrum activity against bunyaviruses, including the viruses causing three important human diseases: Rift Valley fever, Congo-Crimean hemorrhagic fever and HFRS. Hantaan is among the most sensitive RNA viruses to ribavirin *in vitro* and ribavirin therapy decreased the mortality of suckling mice lethally infected with Hantaan. In a prospective, randomized, double-blind, placebo-controlled, clinical trial where patients were administered intravenous ribavirin or placebo, mortality was significantly reduced among ribavirin- compared to placebo-treated patients when comparisons were adjusted for baseline risk estimators of mortality (total serum protein and AST [SGOT]) utilizing a stepwise logistic procedure [p=0.047 (two tailed)]. Treatment initiated by the fourth day of fever showed maximum drug intervention. In this group ribavirin shortened the duration of each post-febrile clinical phase, with significant effects on theduration of hypotensive and oliguric phases, resulting in an earlier onset of the polyuric phase. Treatment initiated on or after the fifth day of fever showed little effect on clinical parameters. The only significant side effect was a reversible anemia. The results of this study show intravenous ribavirin therapy at appropriate doses can provide the first effective drug therapy for early treatment of HFRS.

FC 3-1

MOLECULAR CLONING AND NUCLEOTIDE SEQUENCE ANALYSIS OF THE GENOME OF NEPHROPATHIA EPIDEMICA VIRUS STRAIN HÄLLNÄS B1

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To study the genetic variability of Hantaviruses and identify their antigenic epitopes the M and S of viral RNA segments from nephropathia epidemica virus (NEV) strain Hällnäs B1 were characterized by molecular cloning and nucleotide sequence analysis. Lambda cDNA libraries were established using total cellular RNA isolated from infected Vero E6 cells. To isolate M specific cDNA clones a lambda/gt10 library was sreened by plaque hybridization using probes derived from Hantaan M cDNA clones. To clone the S segment a lambda/gt11 expression library was screened using convalescent phase sera from patients with NE. The nucleotide sequences of M and S cDNA clones were determined and compared to those of Hantaan M and S. A comparative analysis of the deduced amino acid sequences of NEV and Hantaan M reveals a striking similarity of the predicted protein structure even though the homology at the amino acid level is only 53 percent.

This study was suported by BMFT project grant 0318973A.

FC 3-2

CHARACTERIZATIONS OF HANTAAN VIRUS ENVELOPE GLYCOPROTEIN ANTIGENIC DETERMINANTS RELATED TO VIRUS NEUTRALIZATION AND PATHOGENESIS IN SUCKLING MICE.

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A panel of monoclonal antibodies (MAbs) to the G1 or G2 envelope glycoprotein of Hantaan virus was used to characterize the antigenic sites which related to virus neutralization and pathogenicity to suckling mice.

From the results of competitive binding assay and plaque reduction neutralization test (PRNT) using the MAbs, one of the two antigenic sites on G1 and two of the seven sites on G2 were found to relate virus neutralization. One MAb (8E10) showed PRNT titer less than 1:10, but it clearly showed the plaque inhibiting activity with Hantaan and Seoul virus.

Protective effect of MAb to Hantaan virus infection in suckling mice (ICR) was studied by injecting MAb ascitic fluid i.p. four hr. prior to virus challenge (5.0×10^3 ffu, s.c.). Anti G1 (16D2) and anti G2 (HCO2) MAbs completely protected mice from lethal Hantaan virus infection.

FC 3-3

EXPERIMENTAL INFECTION OF HUMAN VASCULAR ENDOTHELIAL CELLS WITH HANTAVIRUSES

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To investigate if hantavirus pathogenicity is dependent on tropism for endothelial cells, we inoculated human vascular endothelial cells with strains of hantavirus isolated from rodent reservoirs captured in geographical regions with and without hemorrhagic fever with renal syndrome. Primary cultures of endothelial cells, isolated from veins of freshly acquired umbilical cords, and an established line of human umbilical vein endothelial cells (HUVEC), maintained in culture as either monolayers or capillary-like structures, were inoculated at a multiplicity of infection of 0.1 with Hantaan virus (strain 76-118), Seoul virus (strain 80-39), Puumala virus (strain Sotkamo) and Prospect Hill virus (strain Prospect Hill I). Endothelial cells were examined for hantavirus antigen by the indirect immunofluorescent antibody technique, at varying intervals for 7 to 10 days, beginning 2 days following inoculation. For all four viruses intracytoplasmic, virus-specific granular fluorescence was detected initially at 2 days postinoculation; at 5 days, nearly 90% of cells contained viral antigen. Specific fluorescence was also detected in infected capillary-like structures growing on Matrigel-coated coverslips. Cytopathic effect or inclusion bodies were not observed. The in vitro demonstration of the susceptibility of endothelial cells to all the hantaviruses studied corroborate in vivo findings, and suggest that endothelial cells may serve as target cells in hantavirus infection. The role of endothelial cell infection in pathogenesis, however, remains to be determined.

FC 3-4

STUDIES ON THE PROPAGATION OF HANTAAN VIRUS 76-118 IN MDCK CELL CULTURES

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This study was intended to establish the susceptibility of Madin and Darby canine kidney(MDCK) cells for the propagation of Hantaan virus and to evaluate the applicability of MDCK cell system for the measurement of fluorescent antibody titers in sera from patients with HFRS.

The results are summarized as follows:

1. On 8 days after Hantaan virus inoculation, specific granular fluorescent antigens developed, exclusively in the cytoplasm of MDCK cells when observed by the IFA technique.
2. Both the percentages of cells exhibiting specific fluorescence and the intensities of such fluorescence increased significantly through the first and second passage of MDCK cells inoculated with Hantaan virus.
3. The growth pattern of Hantaan virus in MDCK cells was fairly similar to those in Vero E6 and A-549 cells. The infectivity titers measured during 2 weeks after virus inoculation were also comparable.
4. Existence of viral RNA in 3 segmented pieces in MDCK cells infected with Hantaan virus was demonstrated in this study.
5. Measurements of fluorescent antibody titers in the use of Hantaan virus infected MDCK cells resulted in the comparable titrations of sera from patients with HFRS. The results of such titrations were in full agreement with those obtained with both A-549 and Vero-E6 cells inoculated with Hantaan virus.

FC 3.6

A PROMISING APPROACH FOR ISOLATION OF HANTAVIRUS FROM HFRS PATIENTS DURING LATER COURSE

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Based on past experience, the success of isolating Hantaviruses(HTV) from patients depended on good quality of blood samples to be collected before or at least on the 5th day of illness. However, HFRS patients in China are usually hospitalized around the 6th day because of several reasons. In order to isolate HTV strains from HFRS in-patients during later course of the disease, peritoneal exudate(PE) from patients with severe form of HFRS was used.

PE was collected from a male, 46-year-old HFRS patient on day 10 and prepared according to the method of Conrad. The isolated PE cells were washed 3 times with Eagle's MEM and then stored in liquid nitrogen until use. Before inoculating on monolayers of Vero E6 cells, the PE cells were subjected to freeze-thawing 3 to 5 times and clarify.

A HTV strain named GH716 was isolated from the 1ot passage on 12th day after inoculation. Further attempt of this isolation was also successful from another male, 44-year-old HFRS patient on 10th day of illness. The specific reaction patterns of these two HTV strains using monoclonal antibodies by IFA were similar to that of the 76-118 prototype. This approach of isolation appears to be of epidemiological significance in tracing dangerous animal hosts in endemic area.

FC 3-6

ISOLATION OF HEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS FROM PERIPHERAL BLOOD LEUKOCYTES OF HUMAN PATIENTS.

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Heparinized whole blood samples were collected from 23 patients with hemorrhagic fever with renal syndrome(HFRS). Luckocytes were separated from the heparinized whole blood by centrifugation over Ficoll/Hypaque density gradient solution(specific gravity 1.070). Serial blind passage of leukocytes with Vero E6 cells was done. 2 HFRS viruses were isolated from the 23 samples. All samples were tested for the immunofluorescent antibodies. Immunofluresecent antibody titer of 2 patient were 1:1024 and 1:256, respectively. The 2 HFRS virus strains were passaged on Vero E6 cell monolyayers.

When nearly 100% of the cells displayed virus specific fluorescence, maximal infectivities were reached peak levels of $10^{6.23}$ and $10^{6.85}$ $TCID_{50}$/ml by day 15 post-inoculation. Simultaneous cross-IFA tests of human sera among the isolated HFRS virus (strain 88-06), Seoul virus (strain 80-39), and Hantaan virus (strain 76-118) revealed different antigenic relationship. 3 consective sera were collected from 14 partients at 2-3 week of intervals, and then compared the antibody titers by simultaneous cross-IFA test. Antibody titers against strain 80-39 was 2-4 fold lower than those against strain 88-06 and strain 76-118. Cross-neutralization test and blocking-antibody test were under the investigation.

PC 3-7

DETERMINATION OF IgM TYPE OF ANTIBODIES AGAINST HANTAVIRUSES IN SERA OF DUTCH AND BELGIAN PATIENTS WITH AN ACUTE FORM OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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Acute and convalescent sera taken 7 up to 500 days p.o.d. from 3 Belgian and 1 Dutch patient with an acute mild form of HFRS were analysed for the presence of Puumala (PUU) and CG 13891 (CG) specific IgM by u-capture enzyme-linked immunosorbent assay (EIA). Both Puumala and CG 13891 viruses were isolated from a bankvole (Clethrionomys glareolus) captured in Finland and Belgium respectively. In parallel, virus specific IgG type of antibodies were determined by the indirect immunofluorescent test (IFT). The EIA IgM values for PUU and CG were strongly positive in sera collected the first month; at 5 - 10 months they were negative or borderline and at 17 months all sera were negative. Only for one Belgian patient EIA IgM for CG was still positive but negative for IgM PUU, 4 up to 10 months p.o.d.. Since IgG antibodies occur often at the time of o.d. but remain present years p.o.d. the presence of IgG antibodies is not significant in the acute phase of the disease. IgM EIA using the local circulating hantaviral antigen becomes a valuable tool for early diagnosis of a mild form of HFRS in low endemic areas.

FC 3-8

DETERMINATION OF IgM TYPE OF ANTIBODIES AGAINST CLETHRIONOMYS (CG 13891) AND APODEMUS TYPE (HNT 76-118) OF HANTAVIRUSES IN SERA O[F] YUGOSLAVIAN PATIENTS WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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Five acute and two convelescent sera of Yugoslavian patients with a more severe form of HFRS did react all in the HNT 76-118 IgM capturing Enzyme Immunoassay (EIA), whereas only one serum (14 %) reacted in the CG 13891 IgG EIA. These sera showed in the indirect immunofluorescent IgG antibody assay (IFA) high titers on HNT 76-118, Fojnica and Dobrava and lower titers on CG 13891 and Vranica (serologic Pattern I).

On a total of 6 acute sera from patients with serologic pattern II (low HNT, Fojnica, Dobrava, high CG 13891, Vranica titers) associated with a mild form of HFRS, five (83 %) did react both in HNT and CG 13891 IgM ELISA.

This was evidence for a serological "one way" cross between Clethrionomys and Apodemus type of induced antibodies of IgM type. Since both viruses circulate in Yugoslavia it is important to distinguish between the two since the prognosis for infected patients will be different. IgM capturing EIA using two different hantaviral antigens will therefore be the method of choice.

FC 3-8

RAPID SERODIAGNOSIS OF HFRS VIRUS INFECTION USING HIGH DENSITY PARTICLE AGGLUTINATION, A PRELIMINARY REPORT

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The antibody against Hantavirus was successfully measured by a method of passive agglutination procedure using high density composite particles (HDP) coated with purified Hantaan virus antigen.

Antigen was prepared from infected suckling rat brain using ultracentrifugation, protamine and ethanol treatment etc.

For preparation of antigen coated HDP, 0.5% HDP suspension was added to an equal volume of antigen and incubated for one hour at room temperature. Then the suspension was washed with PBS, suspended in the diluent containing stabilizers, and then lyophilized.

Microtiter techniques were used throughout all this reaction. To the serial two folds dilutions of sera, every one drop of antigen coated particles were added, and then let stand for more than forty minutes at room temperature.

Positive agglutination patterns using the coated HDP were distinctly demonstrated against positive sera for HFRS antibody while negative ones were found in both negative sera and diluent. It was also found this reaction was more sensitive compared with IFA test.

Accordingly, as coated HDP were lyophilized, this reaction is easily used for measurement of HFRS antibody without any technical complexity, it is expected that this reaction will be apply for clinical use.

FC 3-10

ENZYME-LINKED IMMUNOSORBENT ASSAY USING BACULOVIRUS EXPRESSED NUCLEOCAPSID PROTEIN

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The high level expression of nucleocapsid (N) protein of Hantaan virus was shown using baculovirus recombinant. Expressed protein had been shown to be a useful antigen for diagnosis. We examined the ELISA method using expressed antigen for detection of antibodies in rat sera. Spodoptera frugiperda cells infected with recombinant virus HAN.S were collected, washed, sonicated and dissolved in 0.75% SDS. PBS containing 1% FCS was used for blocking buffer. IFA negative sera of rats captured in Tokyo port area and IFA negative sera from experimental rats were tested by the ELISA method. The ELISA titers of these sera were from <100 to 200. IFA titers of sera which ELISA titers were 400 or more were positive. In antibody positive sera, the ELISA titers were from two to ten times higher than the IFA titers. This ELISA method using expressed antigen was a useful tool for seroepidemiologic study of rat sera. It might be possible to apply this method to another sero- diagnosis including human sera.

FC 3-11

SEROLOGICAL COMPARISONS OF HANTAVIRUS STRAINS

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More than thirty different hantavirus isolates were compared by using various serological procedures in an attempt to define serogroups and explore antigenic relationships of viruses within the genus. Convalescent sera from laboratory rats infected with each of the hantavirus strains were separated into high-titered or low-titered pools for use in the comparison. Monoclonal antibodies described by Arikawa et. al.(1989, J. Gen. Virol.) were also used in the characterization of virus isolates.

Indirect fluorescent antibody testing and ELISA demonstrated extensive cross reactivity among the various strains but allowed the definition of seven separate groups based on different patterns of cross-reactivity. Hemagglutination inhibition testing, using purified virions as hemagglutinating antigen, further defined complexes of viruses within some of the major serogroups. Of the tests examined, neutralization (using screening tenfold dilutions) appeared most specific. This technique allowed further differentiation of the various isolates but also revealed cross-reactions among most members of each serogroup. Immune precipitation of radiolabeled proteins of selected viruses suggested that the nucleocapsid and G2 envelope glycoproteins contain multiple cross-reactive determinants. The G1 glycoprotein reaction was more difficult to observe but this antigen appears more virus- or type-specific.

The definition of Hantavirus serogroups and identification of the antigens responsible for both cross-reactivity and type specificity has significant application to diagnosis and vaccine development research.

Subject Classification
Sero-diagnosis, Oral with Slide Projector

FC 3-12

DIFFERENTIAL SEROLOGIC DIAGNOSIS OF HEMORRHAGIC DISEASES AMONG SUSPECTED HFRS IN KOREA IN 1988.

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Recently acute febrile hemorrhagic diseases that occured in Korea were Hemorrhagic fever with renal syndrome (HFRS), Scrub typhus and Leptospirosis. However, the causes of about a half of patients among suspect HFRS that had been requested the serologic tes. were not identified. Serological studies were performed on 1,216 patients' sera from suspected HFRS and Rickettsiosis in Korea in 1988. The results were summarized as follows. 1) Among the 1,216 patients: 263 HFRS, 215 Murine typhus, 39 Spotted fever group(SFG) rickettsiosis, 98 Scrub typhus and 35 Leptospirosis were diagnosed by microimmunofluorescence test. This is the first demonstration that many SFG rickettsiosis and Murine typhus patients occured in Korea, 1988. 2) The regional occurrences of each disease showed that the Seoul and Kyunggi province occupied about 70% of the patients and the remaining 30% occurred in all over the South Korea. 3) In HFRS and Murine typhus and Leptospirosis, Male patients occupied 72%, 69% and 57% of the patients respectively. In SFG rickettsiosis the number of male and female patients were approximately same and in Scrub typhus, female patients occupied. 4) There is IgG antibody cross reaction in sera from the patients against R. typhi and R. sibirica by the microimmunofluorescence test but IgM antibody was type specific.

FC 3-13

DEMONSTRATION OF PRESENCE OF 5TH SEROTYPE OF HANTAVIRUS: BY INTERPRETATION OF DIFFERENTIAL SERO-DIAGNOSTIC ANALYSES OF SERA FROM HFRS PATIENTS

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Some of sera from patients of hemorrhagic fever with renal syndrome(HFRS) in estern European countries including Yugoslavia, Albania and in Korea recently could be characterized as a new serologic Pattern of HFRS by higher reactivity to Prospect Hill(PH) viral antigen. These serologic characteristic was comparable espicially with so-called Hantaan sero-pattern which is established by the anti-Hantaan virus antisera. Preliminary screening of HFRS sera was carried out by immunoblotting using strips of PH virus. The positive sera were further characterized by differential immunofluorescent antibody and plaque reduction neutralization tests. Among 168 Korean, 15 Yugoslavian and 8 Albanian patients, 6(4%), 6(40%) and 5(63%) sera were found to be a new sero-pattern, respectively. The presence of this pattern was also demonstrable in Apodemus mice sera captured in endemic areas of HFRS in Korea. Interesting features were that the new pattern is happened in Apodemus mice population with more higher frequncy(82%) in an inhabit while that is lower(13%) in another. This results indicate that a new 5th serotype of Hantavirus exists in Korea, Yugoslavia , Albania and this type of virus would be involved in occurance of rather severe HFRS in estern European countries espicially. Characterization of Maaji virus, newly isolated Apodemus virus, as the candidate of 5th serotype is in progress.

FC 4-1

CLINICAL ANALYSIS OF FATAL CASES IN HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS)

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During the last four decades, the case fatality rate was continuously decreased but the exact nature and cause of these changes were not well analyzed. The purposes of this study were to analyze the clinical characteristics and cause of death.

We reviewed 22 fatal cases diagnosed at Seoul National University Hospital which is nationalwide tertiary referral center between 1979 and 1988 by clinical records and findings of six autopsies and necropsies. The overall case fatality rate was 6.3% (22 deaths among 349 patients) with no difference in both sexes. There were one death under age 40(0.75%) and 21 deaths over age 40 (9.77%). The age of fatal cases was significantly ($p=0.003$) older than that of survivors (mean 51.6 $\pm$ 9.0, range 35 to 67 vs 42.3 $\pm$ 14.2, 15 to 78 respectively), this difference was mainly due to male patients and was not evident in female patients.

The causes of death were primary shock, pulmonary edema and hemorrhages, cerebrovascular accidents and sepsis (5 patients in each), and secondary shock (2 cases) with gastrointestinal bleeding.

The number of deaths occurred in the hypotensive,oliguric and diuretic phases were seven,five and ten respectively. Five and twelve patients were expired during the first and the second week of illness respectively. Among the five patients who expired after two weeks of illness, sepsis is the main cause of death in four patients. Eighteen deaths occurred within one week after admission and among these nine deaths occurred within 24 hours after admission.

FC 4-2

SUCCESSFUL DELIVERY IN A PATIENT WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME(HFRS)

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There have been few reports of successful full term delivery in pregnant women who recovered from HFRS requring hemodialysis. Here we report such a case. A 26-year-old woman in the 23rd week of pregnancy was admitted because of oliguria for 1 day. She was well until 5 days prior to admission,when sudden fever,headache followed by vomiting ,lumbago and oliguria developed.

She was a housewife who lived in Seoul. On admission body temporature was 36.3^{o}C,pulse rate 124, respiratory rate 28 and blood pressure was 70/45 mmHg. Multiple petechiae were observed on soft palate and both costovertebral tenderness was elicited.Urine gave 3+ test for protein. Hematocrit was 49.2% ,platelet count 33000 per cubic millimeter, blood urea nitrogen 62 mg/dl and serum creatinine 5.5 mg/dl. Despite of prolonged shock, seizure attack and oliguria of 10 day's duration requring hemodialysis 3 times, she was discharged without complication on the 24th day.

Titers of IgG and IgM antibody to Hantaan virus by ELISA were 1:3,200 , 1:12,800 on the 8th day and 1:25,600,1:12,800 on the 19th day respectively. Titers of IgG and IgM antibody of amniotic fluid was 1:400 and negative respectively on the 15th day. On the 41 weeks of pregnancy she was delivered of a healthy male infant weighing 3.8Kg with Apgar score 10 at 5 min. Titer of IgG and IgM antibody of mother's blood were 1:51,200 and1:3,200 whereas those of cord's and infant's blood were 1:25,600 and negative respectively,which changed to 1:51,200,negative in mother and 1:1,600, negative in infant 4 months later.

Thus we concluded that the above serologic findings are suggestive of not true transplacental infection but passive tranfer of maternal antibody.

FC 4-3

CLINICAL FEATURES OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS) CAUSED BY SEOUL VIRUS INFECTION: A CLINICAL AND LABORATORY STUDY ON 29 CASES IN SEOUL IN 1984.

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Seoul virus is one of the recently identified causative agents of HFRS and isolated from wild urban rats by Lee et al.

Previously several investigators evaluated urban type HFRS, but there are no reports of clinical findings of Seoul virus infection based on serological diagnosis.

We evaluated the clinical findings of 29 patients with HFRS caused by Seoul virus who were diagnosed by hemagglutination inhibition test.

We also compared the clinical findings of Seoul virus infection with previously reported clinical findings of classic Korean hemorrhagic fever (KHF).

The results were as followings ;

1) The disease occurs predominantly in males (80%) with high incidence in 3rd and 4th decade of age.
2) The highest incidence of the disease occurs in October-November.
3) Major symptoms were fever (93.1%), abdominal or flank pain (69%), vomiting (51.7%) and myelgis (41.4%).
4) Major signs were petechine (41.4%), CVA tenderness (41.4%) pharyngeal injection (34.5%) and conjunctival injection (17.2%) but there signs were much less common than in patients with classic KHF.

FC 4-4

MAGNETIC RESONANCE IMAGES (MRI) OF KIDNEY IN HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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One of the major clinical characteristics of HFRS is variable degree of renal insufficiency and pathologic characteristics show the importance of vascular dysfunction and the characteristic hemorrhage in the medulla, especially in the subcortical areas. The purposes of this study were to explore further understanding of hemodynamic vascular events and to explore the capability of MRI for detecting medullary hemorrhage.

We observed the sequential transaxial MRI of kidney in 12 patients with typical clinical course of HFRS and with positive serologic confirmation. Five of those patients had renal biopsies which showed the pathologic findings of HFRS. The biopsies were performed from 4 days before to 3 days after MRI. MRI was performed at 2.0 Tesla(Spectro-20000, Goldstar, Seoul). T1 and T2 weighted spin echo sequences (500 - 600/30 msec, 2000/ 60-80 msec ; TR/TE) were obtained in the axial plane.

Renal shape appeared to be global and the renal sinus fat was compressed in the oliguric phase and these findings reverted to normal as the diseases passed through the late diuretic phase. The striking abnormalities were the prominent corticomedullary distinction with high signal in the cortex and lower signal in the medulla in all 12 cases and band-like very low signal intensity zone at subcortical area which separated the cortex and medulla more clearly on T2 weighted images in all but one patient. These corticomedullary differences were obvious from the oliguric phase , but subcortical low signal band was not always prominent in the early oliguric phase.

We proposed that these findings on MRI might be associated with medullary hemorrhages and subsequent fibrotic changes which were the most severe in the subcortical areas.

FC 4-5

ACTIVATION OF PLASMA KALLIKREIN-KININ SYSTEM IN HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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To investigate the role of kallikrein-kinin system in the pathophysiology of HFRS, we measured plasma prekallikrein, kallikrein-like activity, kallikrein inhibitor by amidolytic assay serially in 20 patients with typical HFRS, and C1 inhibitor by single radial immunodiffusion in eight patients.

Plasma prekallikrein decreased to 36.3 ± 13.3% (mean ± SD) ($p<0.05$) (normal: 93.5 ± 3.2%) in the hypotensive or early oliguric phase and increased gradually to normal value thereafter during the course of illness.

Plasma kallikrein-like activity increased to 3.5 ± 2.6% (normal: 0.4 ± 0.1%) ($p<0.05$) in the hypotensive or early oliguric phase and declined soon to normal value during the course of illness.

Plasma kallikrein inhibitor and C1 inhibitor increased above normal value in oliguric, diuretic, and convalescent phase.

There was a positive correlation ($r=0.76$) between kallikrein inhibitor and C1 inhibitor in patients with activation of kallikrein-like activity.

The clinical characteristics in 11 patients with activation of kallikrein-like activity, include shorter duration from onset of illness to admission, higher scores of clinical severity, higher hematocrit, and lower initial platelet counts.

In conclusion, plasma kallikrein-kinin system was activated in the early phase of HFRS.

FC 4-6

The Changes of Plasma Atrial Natriuretic Polypeptide(ANP) Level According to Each Clinical Phases of Korea Hemorrhagic Fever

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The plasma ANP was repeatedly measured in each clinical phases in 20 patients(20.8±7.2yr) with Korean Hemorrhagic Fever (KHF). The study purpose was to see whether there is any relationship between the clinical course of KHF and the changes of plasma level of ANP.

The plasma ANP level was low(30-80pg/ml) in the early oliguric phase. Then with the sudden remarkable decrease of plasma renin activity and aldosterone the plasma ANP increased rapidly to high level(230-280pg/ml), then was decreased to near normal level(90-190pg/ml) making a narrow peaked plasma ANP concentration curve, and then the level stayed at near normal level during the diuretic phase.

Around the time when the plasma renin activity and aldosterone concentrations were decreased to their low level and the plasma ANP was increased to it's peak level the oliguric phase was changed to the diuretic phase.

FC 4-7

CHANGES IN LYMPHOCYTE SUBPOPULATIONS DURING HFRS

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Distinct populations of immune effector cells often exhibit quantitative changes in response to infection and can be characteristic of a specific viral disease. In order to investigate the immune response to Hantaan virus infection, lymphocyte subpopulations were measured in 15 patients with HFRS. Patients were enrolled in the study on admission to hospital and the diagnosis of HFRS was confirmed by a positive IgM response to Hantaan virus. The subpopulations were measured by flow cytometry with monoclonal antibodies to specific cell-surface antigens which identify helper (CD4+), suppressor (CD8+), total T (CD3+), activated T (CD3+,HLA-DR+), and suppressor/NK subsets (CD8+,Leu7+) of lymphocytes. A three-part white blood cell differential was also calculated for each patient using flow cytometry data. Total white blood cell counts were elevated for most patients with increases in lymphocytes, monocytes, and granulocytes. The most notable increase was observed in the number of monocytes. Within the lymphocytes, both T cells and B cells were significantly increased in early samples. A statistically significant increase in activated T cells was observed in all patients studied and remained elevated beyond the eleventh day of illness. The number of helper cells was at the lower level of the normal range, while suppressor cells were increased in some patients. When the helper/suppressor ratios of all patients were analyzed together, there was an initial depression with a constant increase over time from day 6 to day 24 (first order model, $r=0.9$). No elevation of the CD8+, Leu7+ subset was observed. Of all the parameters studied, the increases in activated T cells and monocytes were the most prominent. These data help characterize changes in the cellular immune response to Hantaan virus infection.

FC 4.8

PLATELET AGGREGATION AND RELEASE IN HFRS PATIENTS

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Hemorrhagic fever with renal syndrome (HFRS), caused by Hantaan virus infection, is characterized by high fever, renal failure, and hemorrhage. Because bleeding manifestations may be the result of platelet dysfunction, HFRS patients were studied to characterize the nature of the platelet defect. Patients were followed from their admission to hospital for up to 15 days. Hantaan virus infection was confirmed by a positive ELISA for virus-specific IgM. The aggregation response (change in light transmission) and the platelet release reaction (ATP-induced luminescence) to the agonists ADP and collagen were measured simultaneously in platelet-rich plasma. Thrombin was used to determine maximum release of granule contents. When tested initially, patients exhibited decreased aggregation and abnormal release reactions, which varied from mild to severe. Platelet function returned to normal with recovery. The platelets from one patient with severe disease were gel-filtered and resuspended in normal plasma to confirm that the defect was not related to a plasma inhibitor. The mechanism of platelet dysfunction in HFRS appears to be variable involving both abnormalities in release and storage of granule contents.

FC 4-9

SCINTIGRAPHIC MEASUREMENT OF THE CHANGES OF PULMONARY VASCULATURE IN KOREAN HEMORRHAGIC FEVER

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Pulmonary vascularity and vascular permeability have been thought to vary according to the disease course of Korean hemorrhagic fever (KHF), and the extreme changes of these variables might lead to the fatal outcome among the most severely ill patients. We adopted radiolabelled albumin cinescintigraphy which had been applied for the the evaluation of the adult respiratory distress syndrome with permeability edema, and tried to assess the changes of pulmonary vasculature in KHF.

In 10 patients, sequentially in 7 patients, we performed lung and heart cinescintigraphy with ^{99m}Tc albumin and acquired the curves of density ratios for the selected regions of interests: heart, lungs and liver. We took the density ratio of lung-to-heart and that of liver-to-heart at 20 minutes after the albumin injection for an index reflecting pulmonary and hepatic blood volumes respectively. We considered the density ratio changes (represented by the slopes of the curves) of the lungs and the liver between 20 and 50 minutes as another index reflecting vascular permeability.

Density ratios of lungs-to-heart and liver-to-heart taken during late phases of Korean hemorrhagic fever tended to aggregate near the value of 0.5. Density ratios of lungs-to-heart ranged between 0.35 and 2.0, and these ratios in the oliguric period just after the hypotensive phase increased upto 2.0 and then tended to decrease. Density ratios of the liver-to-heart ranged between 0.5 and 3.0, and showed the same pattern of changes as that of the lungs.

The slopes of the lungs representing the tidal changes of the density ratios ranged between 2.3*10E-2 and -3.8*10E-2. The curves of lungs-to-heart in the initial phases of KHF showed positive deflection before and even after the hypotensive phase. 4 among 24 images showed these findings. During the oliguric period after hypotensive phase, the curves showed negative deflection, which at last recovered their flatness at a later period. Beginning to come to zero just after the hypotensive phase in a few cases, the slopes were nearly null in most cases late at diuretic phase. The slopes of liver-to-heart ranged between 3.0*10E-2 and -5.6*10E-2. The general tendency of the changes was the same as those of the lungs, but the amplitude of the variations seemed larger.

We could find that radiolabelled albumin cinescintigraphy reflected the variably changing features of pulmonary vasculature. It was concluded that pulmonary vascular permeability was increased at the early phases in some patients with KHF and that pulmonary blood volume was increased at the later period before normalization.

FC 4-10

DISTINCTIVE INCLUSION BODY IN THE HEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS-INFECTED RATS AND CULTURE CELLS

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The morphology of hemorrhagic fever with renal syndrome (HFRS) virus-infected rats and culture cells was examined by immunofluorescence and immunoelectron microscopy. New born F344/JCL rats and monolayers of Vero E6 cell were infected with B-1 strain (Osaka University) of HFRS virus. 3 weeks after infection, the rats and cells were processed for immunostaining using B-1-80A monoclonal antibody (Osaka University) for HFRS virus. Normal rats and uninfected culture cells served as controls. Immunofluorescence microscopically, the monoclonal antibody was positive in the rats and culture cells. The immunoreactivity for the antigen was intensive in the cells of cerebral hipocampus and the kidney convoluted tubule and lung infiltrated cells and faint in the cerebro-cortical cells. The culture cells retained an intensive positive reaction. No positive reaction was observed in the control specimens. Electron microscopically, pleomorphic inclusion bodies with tubular-filament lattice were identified in the virus infected rat and culture cells. The body appeared to consist of numerous filaments, approximately 5 nm in diameter and 2-3 μm in length. The present immunoelectron microscopy indicated an intensive positive reaction of B-1-80A antibody on the inclusion body. These data suggest that the body is important for a maker of HFRS-virus infection.

FC 4-11

PROSPECTIVE STUDY ON PANHYPOPITUITARISM AS A SEQUELA OF HEMORRHAGIC FEVER WITH RENAL SYNDROME (HFRS)

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In HFRS, caused by Hantavirus, pituitary necrosis is a characteristic and common autopsy finding. Contrary to this, only 14 cases of clinical panhypopituitarism have been reported. Recently we confirmed six patients with panhypopituitarism by high resolution sella computed tomography(CT) and combined anterior pituitary stimulation test(CAPST) among 255 patients with HFRS admitted to the Seoul National University Hospital (SNUH) from 1979 to 1986.

So we performed a prospective study on clinical panhypopituitarism as a sequela of typical oliguric HFRS by CT and CAPST in 27 patients, who were admitted to the Dept of IM at SNUH from August 1987 to August 1988. Among 27 patients, 17 showed abnormal CAPST. Among 23 patients on whom high resolution CT was performed, severe pituitary abnormalities were found in six patients and mild abnormalties were found in four patients. Other 13 patients showed no abnormality.

Six patients whose CT findings were severe abnormal, all had abnormal CAPST as well. In four patients whose CT showed mild abnormality, two had abnormal test. In 13 patients whose CT showed no abnormality, eight patients had normal and five had abnormal CAPST. And four of these five patients with abnormal CAPST had impaired renal function.

Three out of six patients who had abnormalities in both CT and CAPST had panhypopituitarism clinically.

Conclusively, panhypopituitarism is considered to be one of the important sequelae of HFRS.

FC 4-12

DYNAMIC OBSERVATION OF BLOOD SYSTEM CHANGES IN EPIDEMIC HEMORRHAGIC FEVER.

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Detections of blood system were carried out in 95 epidemic hemorrhagic fever (EHF) patients at all stages. It was found that each of items had some changes at all stages. Platelet counts began to decrease at febrile stage. It was lowest at hypotensive stage than that of other stages. It became recovery at diuretic stage. White blood cell counts began to increase at febrile stage. It was markedly higher at hypotensive stage than that of other stages. It returned to normal at diuretic stage. The total lymphocytes, atypical lymphocytes, monocytes, band neutrocytes increased and the segmented neutrocytes decreased at each stage. RBC, HGB, HCT increased at febrile and hypotensive stages. HGB, HCT decreased at oliguric, immigrant and diuretic stages. HGB was still lower at convalescent than that of normal value. MCV had some decrease at each stage except convalescent. MCH and MCHC had some decrease and RCMI and RET had some increase at each stage.

FC 4-13

DETECTION AND CLINICAL SIGNIFICANCE OF CREATINE PHOSPHATE KINASE ISOENZYME IN EPIDEMIC HEMORRHAGIC FEVER.

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This paper reports the results of detection and analysis of creatine phosphate kinase isoenzyme (CK-MB) on 78 epidemic hemorrhagic fever (EHF) patients. Data obtained showed the CK-MB content increased in the early stage of disease (at febrile stage or on the fourth illness day). Its high peak appeared at hypotensive and oliguric or on the sixth illness day. It returned decrease at diuretic or on the tenth illness day and recovered to normal level at convalescent or on the eighteenth illness day. It appeared that the change of CK-MB was more severe at hypotensive and oliguric than that of other stages. This seemed to indicate that EHF patients had heart damage.

HFRS: END OF THE BEGINNING. BEGINNING OF THE END?

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Since the original isolation of Hantaan virus just 13 years ago, there has been explosive growth of knowledge concerning almost every aspect of the diverse natural elements which lead to HFRS in man. We have identified at least 4 clear virus immunotypes, documented that these agents form an independent genus among the Bunyaviridae, and come far in understanding molecular structure and function of these agents. We have reasonable tools for virus isolation and sero-epidemiology, and they have been used to reveal a worldwide pattern of rodent and human infection both indoors and out. We have added to knowledge concerning human disease pathogenesis and rodent-virus biology and ecology, and even achieved the first step in specific antiviral therapy. Although new chapters remain, it is fair to say now that a major book entitled Natural History has been written.

Are we ready for definitive intervention in the form of one or more Hantavirus vaccines? The agents themselves seem to be fair game; honest fellows who do not play dirty tricks by compromising host immune systems or changing antigenic raincoats every year or every month. Indeed, the first inactivated products have been administered to man. My main purpose is to assess both the resources and the problems which must be dealt with in order that a second book entitled Disease Prevented might be written.

DIFFERENT PATTERNS OF SPECIFIC SERUM IgG ANTIBODY RESPONSE IN NEPHROPATHIA EPIDEMICA (NE) PATIENTS

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Acute and convalescent serum samples from 74 patients with symptoms suggestive of NE were analysed for the presence of specific IgG antibodies to Puumala virus using indirect immunofluorescence technique (IFAT). Twenty-five (34%) of the patients had ≥4-fold IgG titer changes or seroconversions, while 46 (62%) had persisting high titers (≥1:640). Three patients had titers ≥1:160. Seroconversions were found exclusively in patients where the first serum sample had been drawn within one week after onset of symptoms. Significant titer changes (≥4-fold) were recorded only in patients bled within 8 days after onset of symptoms. These findings indicate that clinical symptoms in NE could be the result of an immunological reaction to Puumala virus, rather than being a direct effect of the infecting virus. Sera from 27 patients were in addition analysed for the presence of IgG antibodies against the antigenically related Hantaan virus using IFAT. Serum IgG titers were ≥8-fold higher when using Puumala virus as antigen compared to Hantaan virus. Sera from 5 patients were non-reactive in the Hantaan serology, while anti-Puumala IgG titers were ≥1:640. This might indicate the existence of different strains of NE virus, showing varying cross-reactivity with the Hantaan virus.

PREPARATION OF HFRS IMMUNOGLOBULIN AND STUDY OF ITS VIRUSNEUTRALIZING ACTIVITY.

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Production lots of immunoglobulin were prepared from HFRS convalescent sera and plasm in European foci. Antibody to all HFRSV serotypes were detected in IFA. Titre with homologous antigens were: 1113 C.g., Blood B strains - 1:640 ; with heterologous HNT, PHV - 1:160, TCH - 1:40. The neutralization test revealed antibody titre to European strains (Cl.glareolus) and to Far-Easter strain (M.fortis) - 1:417 - 1:320 respectively. Antibody titres (NT) to strains from A.agrarius (Far East) were 1:16 - 1:32. Antibody titres in convalescent sera (Far East) to European strains were minimal.

The treatment of bank voles with immunoglobulin (1hr after i/m inoculation with 1000 LD_{50} of Blood B strain) resulted in 100% protection. The susceptibility of bank voles to HFRSV markedly decre-ased when immunoglobulin was administrated 7 days postinfection.

ISOLATION OF HANTAVIRUS FROM SPINAL FLUID OF HFRS PATIENT AND STUDY OF THE NATURE OF THE VIRUS.

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During the epidemic of HFRS in our city a patient showing severe central nerve symptoms was hospitalized with preliminary diagnosis of meningitis. On the day of admission, 13th day of illness the spinal fluid shown to be a clear fluid with one plus protein and some amount of W.B.C. On the 18th day of illness the spinal fluid shown to be a entirely normalfinding. Because of the reason that this patient coming from endemic area and had a history of few days fever HFRS was suspected. Therefore the spinal fluid was tested for Hantavirus antigen by precipitation test with HFRS immune serum. As the test shown strong positive reaction, the spinal fluid was inoculated to Vero-E6 cell culture. In the second passage Hatanvirus particle was seen in the cultured cells.

The virus wassystematically studied and found to be a wild type of Hantavirus similar to 76II8 strain of Hantavirus and no particularvirulence was observed in suckling mice by any routes. The cause of severe central nerve symptoms may be attributed to the persistance of virus in the nerve cells. It may be suggested that in case of HFRS patient with severe central nerve symptoms, examination of spinal fluid for Hanta virus may be helpful for dignosis.

OBSERVATION OF HFRS EPIDEMIC IN AN INSTITUTE CAUSED BY INFECTED LABORATORY ANIMAL.

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In an institute situated in HFRS endemic city, 22 persons contracted HFRS during two years. The cause of the infection has been proven to be the contaminated white rat stock colony where the infection rate of rats by Hanta virus is 23.8%.

During two years 6 laboratories bought white rats from the stock colony and bred them in their laboratory for some times such as 1-3 month before examination for virus infection. In all 6 laboratories laboratory workers contracted infection without exception. In one laboratory 6 workers contracted infection where their animal infection rate is 77.7%, which may be due to cross infection among inbred rats during breeding in their laboratory.

Clinically, most of the patients were mild and no motality what so ever. In some cases the clinical symptoms were so mild only few days low grade fever that the diagnosis was made only by serological examination.

PATHOGENICITY OF EPIDEMIC HEMORRHAGIC FEVER VIRUS ISOLATED FROM PATIENTS ON SUCKLING MICE.

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Having found the antigen difference between the birus isolated from patients and the virus isolated from A.agrarius in Hubei epidemic area, we studied the virus infection of suckling mice. 2-4-day-old mice were inoculated intracerebrally. They began to be ill in the 12-14 day and died within 2-4 days after signs of illness were found. The virulence of virus on suckling mice and yields of virus increased in mice brain with successive passage, as evidenced by increasing TCID50, mice ID50 and LD50. The infective organs and infective cells in the same organ also increased. In addition, we observed the kinestic distribution of virus antigen in suckling mice intraperitonelly. The virus was first isolated from their abdominal macrophage after inoculation 6hrs, then from the bloc in second day. The virus antigen was first observed in lungs (the 4th day) and brains (the 5th day) then appeared in hepars, thymus, spleen and kidney. The Ag increased with the time of inoculation in lungs and brains. Specific EHF virus fluorencent antibody was first detected in the 10th day with the titre of 1:10 and reached the highest level in the 17th day with the titre of 1:640. The mice died in the 18th day.

INTERRUPTED STUDY OF VIREMIA OF PATIENTS WITH HEMORRHAGIC FEVER WITH RENAL SYNDROME IN FEBRILE PHASE.

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Kinetic change of viremia were observed in 287 cases of patients with hemorrhagic fever with renal syndrome (HFRS) which ribavirin was admistered with a double blind random controlled studied by means of virus, indirect immunofluorescence technique and ELISA, in order to confirm inhibition effect of ribavirin to Hantan virus in the patients with HFRS. Positive rate of viremia is 79.7%, positive rate of HFRS IgM is 85.0%. Viremia of patients with HFRS could be interrupted by ribavirin, that is, ribavirin group compared with placebo group, positive rate of viremia is decreased, viremia desappears 8 days after treatment in ribavirin group but 17 days in placebo group in all patients. The average persistent time of viremia was shorter in ribavirin group (3.6 days) than the placebo group (6.9 days). Virus titers and viral antigen products were reduced in ribavirin group. Results above showed that viremia was very frequent in the patients with HFRS in febrile phase, ribavirin is certainly effective drug for the treatment HFRS.

PREPARATION AND APPLICATION OF PIG SPECIFIC TRANSFER FACTOR AND IRNA OF JR-1, JR-2, JR-3 VIRUS STRAINS OF EHF WITH NEPHROTIC SYNDROM. Han Hou Zhe, zu chang yu, ren dong xian, jin chang fan et al, Yan Bian Medical College, Jilin Province, People's Republic of China.

1, 9 strains of apodemirs type of EHF virus were isolated from organs of infected rabbits which were entrapped at the EHF endemic area in Yan Bian of Jilin Province in 1986 by not only intracerebrally into suckling mice and passage but also culture and passage in Vero E6 cell. 3 strains of them were systematically identified. The results shows that these strains are characterised as short latent period, strong immunogenicity and strongtoxicity.

2, The two group virus of activation and inactivation by heating were used as antigen for several immunity at multiple place in 6-month-old pig. The spleen and liver of immunised pig were taken when the serum fluoresecence antibedytiter was above 640. The EHF virus with nephrotic syndrome of specific transfer factor was prepared with pigs spleen by routine way and keeping in freeze drying. It contains 3 unit per ml.. The liver of pig was used for preparation IRNA by chemical way and keeping in freeze drying. It contains 1 mg per ml..

3, These preparations were introduced to climical observation after the examination and the permission. The results shows that "three pain", "three red", atypical lymphcyte, proteinuria and WBC disappeared fast as well as the platelet recovered early. These also can recover severe pation's symptoms and increasing the rate of emergency treatment success. In a word, these preparations can decrease death rate, lighten the state of illness and shorten the course of disease for EHF pations but no side effect.

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